3-Methoxyisoprenaline: a potent selective Uptake$_2$ inhibitor

Burgen & Iversen (1965) investigated the structural requirements within β-phenethylamine derivatives for inhibition of uptake of noradrenaline by Uptake$_1$ and Uptake$_2$. They obtained an estimate of the affinity (based on the concentration of derivative required to obtain 50% inhibition of noradrenaline uptake (IC 50)) of each derivative for each uptake site relative to that of phenethylamine. They summarized their results as: β-hydroxylation, *N*-substitution by a methyl or isopropyl moiety and particularly 3-methoxylation of phenethylamine enhanced the affinity of the compound for Uptake$_2$ whereas the affinity for Uptake$_1$ was in general reduced. However, normetanephrine was more selective than metanephrine in inhibiting Uptake$_2$ relative to Uptake$_1$.

We predicted that the 3-*O*-methylated metabolite of isoprenaline, 3-methoxyisoprenaline, which carries all three substitutions said to enhance affinity for Uptake$_2$ and in general reduce it for Uptake$_1$, would show greater inhibition of Uptake$_2$ than normetanephrine (and perhaps metanephrine) and may also be more selective in inhibiting Uptake$_2$ relative to Uptake$_1$.

The inhibition of Uptake$_1$ and Uptake$_2$ by normetanephrine and 3-methoxyisoprenaline was measured using the perfused isolated heart of the mouse.

Male Swiss mice were stunned and killed by decapitation. The heart was removed and perfused by the Langendorff technique, using a double coil preheating column with a common outlet cannula. Krebs-Henseleit solution, bubbled with 5% carbon dioxide in oxygen and warmed to 37° perfused the heart at a constant flow rate of 1 ml min^{-1}. Hearts were perfused with Krebs solution (with or without normetanephrine or 3-methoxyisoprenaline) for 5 min before perfusion with the same solution containing [^{3}H]noradrenaline (0·059 μM or 29·6 μM) or [^{3}H]isoprenaline (12 μM). The concentration of radioactive label was 12 nCi ml^{-1}.

The initial rate of uptake of noradrenaline at the lower concentration (Uptake$_1$) was estimated by perfusing the heart for 5 min with [^{3}H]noradrenaline followed by a 3 min wash out. The radioactive label was extracted by homogenization in 0·4N perchloric acid solution, and a 1 ml sample taken for radioassay. When noradrenaline at the higher concentration, or isoprenaline, was perfused, the initial rate of uptake (Uptake$_2$) was estimated by measuring the arterio-venous differences across the heart at 1 min intervals during the first 3 min of perfusion with the labelled catecholamine. In this case a correction was made for the [^{3}H]catecholamine in the extracellular fluid space. The apparent volume of the extracellular fluid space determined using either [^{14}C]sorbitol or [^{3}H]noradrenaline in the presence of normetanephrine (10^{-3}M) and cocaine (10^{-3}M) was 0·23, 0·32, 0·35 ml g^{-1} of tissue at 1, 2 and 3 min respectively (Mireylees, 1972).

From the initial rate of cellular uptake in the presence of normetanephrine or 3-methoxyisoprenaline the percentage inhibition of control uptake was calculated by using the equation:

$$\% \text{ inhibition} = \frac{\text{control rate} - \text{rate in presence of inhibitor}}{\text{control rate}} \times 100\%$$

Log concentration-inhibition curves were drawn (Fig. 1) and the IC 50 of each inhibitor for each substrate found from the straight regression line calculated by the method of least squares. Concentrations of inhibitor causing very large and very small degrees of inhibition were avoided. In all statistical analyses critical probability was taken as $P = 0{\cdot}05$.

Normetanephrine and 3-methoxyisoprenaline showed concentration-dependent inhibition of the initial rate of cellular uptake of noradrenaline at both the lower and higher concentrations and of isoprenaline. The IC 50s of the inhibitors against the

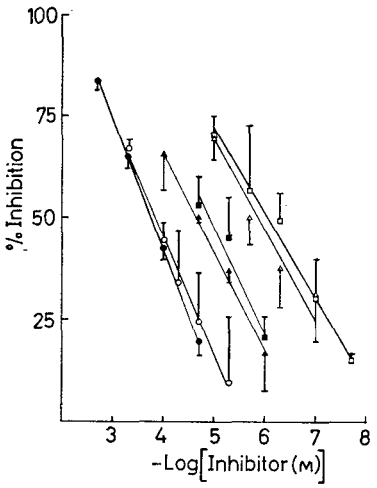

FIG. 1. Log concentration (M): effect curves of the % inhibition of the initial rate of uptake of [^{3}H]catecholamines produced by normetanephrine and 3-methoxyisoprenaline. Inhibition of uptake of noradrenaline (0·059 μM) by normetanephrine (●) and 3-methoxyisoprenaline (○). Inhibition of uptake of noradrenaline (29·6 μM) by normetanephrine (■) and 3-methoxyisoprenaline (□). Inhibition of uptake of isoprenaline (12 μM) by normetanephrine (▲) and 3-methoxyisoprenaline (△).

substrates are summarized in Table 1. The two inhibitors were equipotent in inhibiting Uptake$_1$ but 3-methoxyisoprenaline was more potent than normetanephrine in inhibiting Uptake$_2$.

The concentrations of noradrenaline used were the same as those used by Burgen & Iversen (1965). Uptake of the label was used as an index of catecholamine uptake and is believed to be valid since uptake of catecholamines occurs before metabolism.

When Uptake$_2$ was estimated using noradrenaline at the higher concentration, no allowance was made for noradrenaline which had been taken up by Uptake$_1$; its contribution to the total should be less than 20% (Iversen, 1963, 1965; Iversen, Salt & Wilson, 1972). The pattern of inhibition of the uptake of noradrenaline at the higher concentration by 3-methoxyisoprenaline and normetanephrine was similar to that of the inhibition of the uptake of isoprenaline by these inhibitors. These two patterns were different from the pattern of inhibition obtained against noradrenaline at the lower concentration. Since isoprenaline is only removed by Uptake$_2$ this supports the contention that the inhibition of the initial rate of uptake of noradrenaline at the higher concentration is indicative of inhibition of Uptake$_2$.

3-Methoxyisoprenaline is 14 times more potent than normetanephrine in inhibiting the uptake of noradrenaline by Uptake$_2$ in the mouse heart and 16 times more potent in inhibiting the uptake of isoprenaline. It is also 11 times more selective in inhibiting

Table 1. *The IC 50s of normetanephrine (NMN) and 3-methoxyisoprenaline (MOXA) for the inhibition of the initial rate of cellular uptake of* [^{3}*H*]*noradrenaline (NA) (0·059 μM and 29·6 μM) and of* [^{3}*H*]*isoprenaline (IP) (12 μM). Mean IC 50s were compared and * between values indicates that they were* not *significantly different (P > 0·05).*

	NA (0·059 μM)	NA (29·6 μM)		IP (12 μM)
NMN	175	11·7	*	21·8
MOXA	144*	0·85	*	1·34

$Uptake_2$ relative to $Uptake_1$. This is because 3-methoxyisoprenaline is 169 times, while normetanephrine is only 15 times, more potent in inhibiting $Uptake_2$ than $Uptake_1$.

The results of Burgen & Iversen (1965) show that in the rat heart normetanephrine was 48 times, while metanephrine was 15 times, more potent in inhibiting $Uptake_2$ than $Uptake_1$. The IC 50 for normetanephrine on $Uptake_1$ was $1{\cdot}75 \times 10^{-4}$ M in this study which is similar to the value of 2×10^{-4} M obtained by Burgen & Iversen (1965) in the rat heart. Jarrot (1970) has shown that the affinity of noradrenaline for the neuronal uptake site in the perfused mouse heart is similar to that in the rat, so it seems reasonable to combine the results of Burgen & Iversen with those of this study in order to obtain an idea of the selectivity of metanephrine relative to normetanephrine and 3-methoxyisoprenaline. This suggests that the order of increasing selectivity for $Uptake_2$ in preference to $Uptake_1$ is metanephrine $<$ normetanephrine $<$ 3-methoxyisoprenaline.

The greater selectivity of 3-methoxyisoprenaline for $Uptake_2$ than $Uptake_1$ may allow its use as a pharmacological tool in experiments in which inhibition of $Uptake_2$ is required but $Uptake_1$ must be relatively uneffected.

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Monoamine tetrazolium reductase of rat heart mitochondria

The presence of monoamine dehydrogenase (MADH) in rat brain and liver suspensions has been reported earlier through the use of tetrazolium salts as hydrogen acceptors (Lagnado & Sourkes, 1956). Studies indicating a dissimilarity of rat brain MADH from monoamine oxidase (Guha & Ghosh, 1970) led us to investigate the presence of MADH in rat heart mitochondria and study some of its properties.

Heart homogenate and isolated mitochondria from albino rats (200–300 g) were prepared in cold 0·25 M sucrose (Sen, Parmar & Guha, 1968). The reaction mixture for determination of MADH activity consisted of 0·025 M tris-HCl buffer, pH 8·0, 0·5 mg neotetrazolium chloride (NTC), 0·01 M of the desired substrate and tissue homogenate or isolated mitochondria equivalent to 100 mg wet tissue weight in a total volume of 2 ml. The reaction mixture containing heart mitochondria was incubated at 37° for 10 min. After the addition of the desired substrate, the mixture was further incubated for 30 min using air as a gas phase for aerobic conditions whereas anaerobic experiments were carried out in a vacuum in Thunberg tubes (Guha & Ghosh, 1970). MADH activity was determined by estimating at 520 nm the red diformazon formed as a result of NTC reduction (Lagnado & Sourkes, 1956). Monoamine oxidase